Brucella spp. Specimen Selection

- Serum
  - The diagnosis of brucellosis is frequently achieved by serology. An acute & convalescent phase specimen should be collected (21 days apart)
- Blood or bone marrow
  - Brucellae most often isolated from these
- Tissue (spleen, liver)
  - Brucellae occasionally isolated

Brucella spp. Biosafety Alert

+ **Brucellosis is THE most commonly reported laboratory-associated bacterial infection.**

+ **Cases have occurred in clinical laboratory settings by “sniffing” cultures, direct skin contact with cultures, and aerosol generating procedures**

Level A Laboratory Tests *Brucella* spp.

+ Colonial morphology on SBA
+ Gram stain morphology
+ Oxidase
+ Urea hydrolysis
+ BSL 3

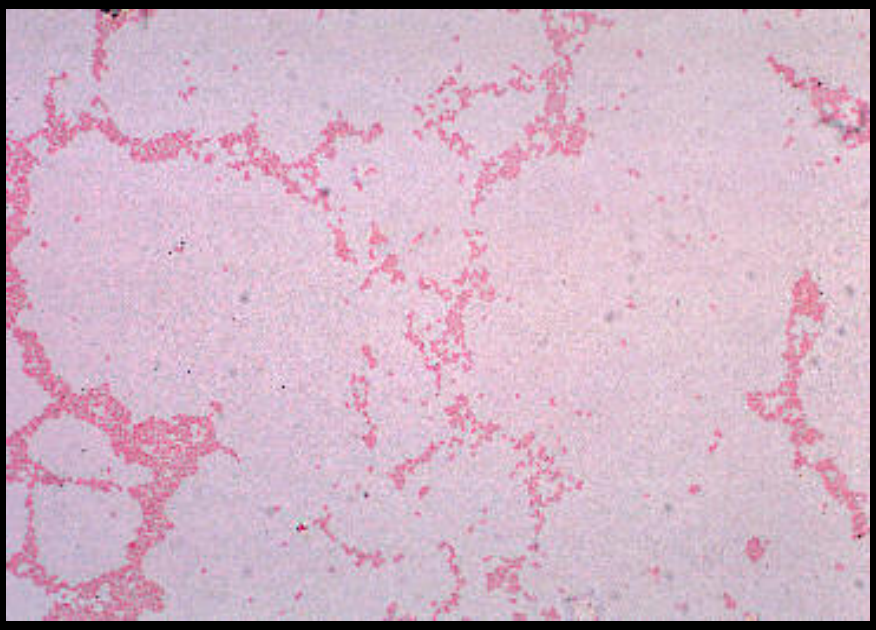
Brucella spp. Key Level A Lab Tests

+ Colonial morphology on SBA
  - Fastidious
  - Visible growth may take 48 - 72 hrs
  - Small (0.5-1.0mm), convex, glistening
  - Non-hemolytic and non-pigmented

Brucella spp. Key Level A Lab Tests

- Gram Stain Morphology
  - Tiny (very) - 0.5 - 0.7? x 0.6 - 1.5?
  - Faintly staining
  - Gram-negative coccobacilli
- Technical Hints
  - B. melitensis: the most coccal (rarely >1?)
  - B. abortus: may be rod-like (2 - 3 ?)

B. abortus, gram stain, (x2400)



B. abortus, gram stain, (x3200)

Brucella spp. Key Level A Lab Tests

+ Oxidase-positive
  - *B. melitensis* (100%)
  - *B. abortus* (96%)
  - *B. suis* (95%)
  - *B. canis* (72%)
+ Urea hydrolysis-positive
  - *B. suis & B. canis* ~15 min
  - *B. abortus & B. melitensis* ~24hr

Brucella spp.
Other Ox+/Ur+ GN Oxidizers

+ Achromobacter grp B
+ Acidovorax spp
+ Agrobacterium spp
+ EO-2/EO-3
+ Flavobacterium spp
+ Methylobacterium spp
+ Ochrobactrum
+ Pseudomonas spp
+ Riemerella
+ Roseomonas spp
+ O-2
+ II-i

Brucella spp. Rapid Method Results

+ Dade Behring
  - MicroScan Conventional Panels:
    - Currently, not in data base
  - MicroScan Rapid Panels:
    - Currently, not in data base
+ Biolog
    - Currently, not in data base

Brucella spp. Rapid Method Results

+ bioMérieux
  - API 20E: Currently, not in data base.
    - At 48 hrs with only oxidase +, “Low discrimination” with a note: possible Brucella spp.

Brucella spp. Rapid Method Results

Vitek GNI + Card: Not in data base.

- ***B. abortus*: Nonfermenting GNB (asaccharolytic) or *Chryseobacterium indologenes/Brevundimonas vesicularis* or Questionable Biopattern**
- ***B. canis: Acinetobacter* urea or Nonfermenting GNB/Acinetobacter urea**

Brucella spp. Rapid Method Results

+ bioMérieux (cont'd)
  - Vitek GNI + Card: Not in data base
    - *B. melitensis*: Nonfermenting GNB (asaccharolytic) *Flavobacterium odoratum* GCMS (Good Confidence Marginal Separation)
    - *B. suis: Ochrobactrum anthropi* or unidentified
  - Vitek 2 GNB Card: IN THE DATA BASE
    - *Brucella spp.*: True accuracy not yet determined

Brucella spp. Technical Hints

- **Misidentified as *Moraxella sp.***
  - **Clin Inf Dis 1993; 17:1068-9**
- Reported as gram-positive cocci, mistaken for “slow-growing” *Staphylococcus sp.*

Brucella spp. Review of Key Tests

+ Tiny, faintly staining, gram-negative coccobacilli from blood or bone marrow
+ Slow growth on SBA, 2-3 days for colony appearance
+ Oxidase +
+ Urease +

Clostridium botulinum

Botulism

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

BOTULISM

+ The microbe, *C. botulinum,* was first described in 1897 by E. van Ermengem following a foodborne outbreak in Belgium

+ Botulism is a neuroparalytic illness resulting from the action of a potent toxin

BOTULISM

- The diagnosis of botulism is made clinically, i.e., based on the patient's case history and physical findings
- Health care providers suspecting botulism should contact their State Health Department Epidemiology Office

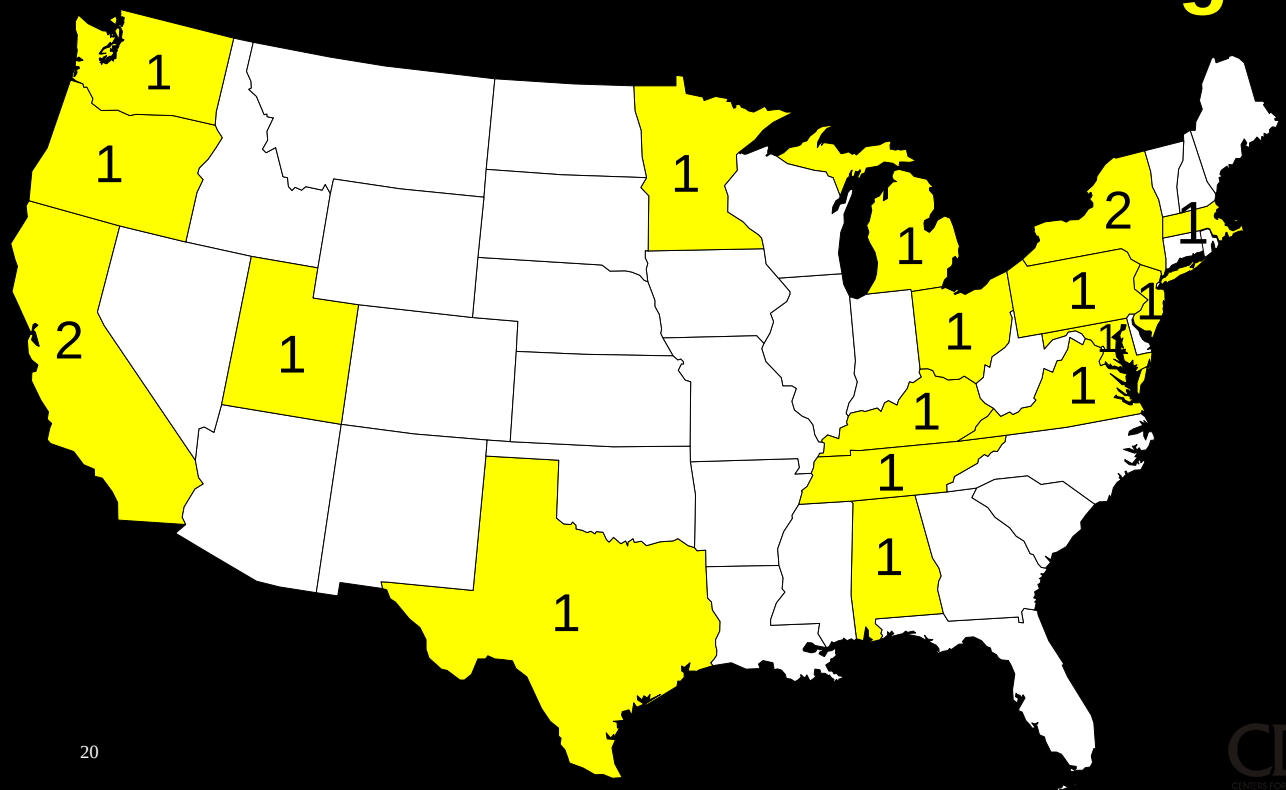
BOTULISM

+ The Foodborne and Diarrheal Diseases Branch of CDC provides emergency consultation and support to public health authorities

+ Days: 404-639-2206,M-F,0830-1630 EST

+ Nights/weekends:404-639-2888

Laboratory Capacity for Botulinum Toxin Testing



CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

BOTULISM

+ There are seven types of *botulinum* toxin:
  - Types A, B, E and in rare cases, F causes disease in humans
  - Types C and D cause disease in birds and mammals
  - Type G, identified in 1970, has yet to be confirmed as a cause of illness in man or animals

FOODBORNE BOTULISM

- Infective dose: 0.001 ?g/kg (type A, LD_{50})
- Incubation period: 18 - 36 hr (6hr to 10 d)
- Dry mouth, double vision, droopy eyelids, dilated pupils
- Generalized, progressive descending bilateral muscle weakness & paralysis
- Respiratory failure and death
- Mortality 5-10%, up to 25%

FOODBORNE BOTULISM

- Among 309 persons with clinically diagnosed botulism reported to CDC from 1975 to 1988:
  - Stool cultures for C. botulinum: 51% +
  - Serum botulinum toxin testing: 37% +
  - Stool botulinum toxin testing: 23% +
- Overall, at least one of the above tests was positive for 65% of all patients

Level A Procedures for Botulism Event

- Properly collected specimens are to be referred to designated testing laboratories
- Prior to the shipment of any botulism-associated specimen, the designated laboratory must be notified and approved by the State Health Epidemiology Office

Level A Procedures for Botulism Event

+ Clinical specimens to be collected:
  1. Serum
  2. Gastric contents or vomitus
  3. Feces or return from sterile water enema
  4. Food samples
+ Autopsy specimens:
  1. Serum
  2. Gastric and intestinal contents

Botulism Biosafety Alert

+ Botulism toxins are extremely poisonous

+ Minute quantities acquired by ingestion, inhalation, or by absorption can cause profound intoxication and death

+ All materials suspected of containing toxin must be handled with CAUTION!

Botulism Biosafety Alert

+ Materials suspected of containing botulism toxin must be handled:
  - **Biological Safety Cabinet (Class II)**
  - **Laboratory Coats**
  - **Disposable surgical gloves**
  - **Face shield (as needed)**

Botulism Clinical Hints

+ The specimen of choice for confirming the diagnosis of botulism:
    1. Serum toxicity testing
    2. Cultures* of debrided tissue from wound
    3. Cultures* of stool or incriminated food(s)

    *confirm isolates are toxigenic

Bacillus anthracis

Anthrax

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

ANTHRAX

+ Three forms of human anthrax occur:
    1. Cutaneous
    2. Gastrointestinal
        - Oropharyngeal
        - Abdominal
    3. Inhalation (Woolsorter's Disease)

Cutaneous anthrax

Vesicle development, day 2

Eschar formation, day 4

Anthrax Lesion on Neck

Inhalation Anthrax

- Infective dose = 8,000 - 15,000 spores
- Incubation period = 1-6 days
- Duration of illness = 3-5 days
- Fever, malaise, and fatigue
- Short period of improvement = up to 2 days
- Abrupt respiratory distress…death <24hrs
- Person to person transmission = no

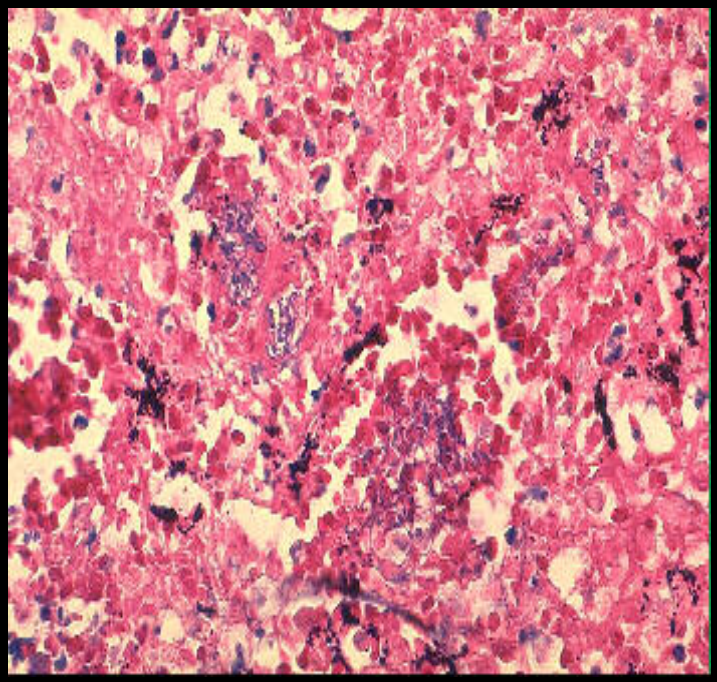
Inhalation Anthrax

+ Chest x-ray is often pathognomonic: widened mediastinum with pleural effusions, without infiltrates.

+ Late in the course of infection, gram stain of blood may reveal broad gram-positive rods.

+ Only vegetative, encapsulated bacilli are present in tissues during infection, typically spores are not seen

Mediastinal LN, microcolonies of *B anthracis*, Giemsa stain



Laboratory Handling of *Bacillus anthracis*, BSL 2

- Anthrax vaccination is NOT required
- Laboratory coats and gloves must be worn
- Safety glasses/shields are recommended
- No hand/finger contact with mucosal surfaces
- Hand washing before leaving lab area

Anthrax: Specimen Selection

+ Inhalation: Sputum and Blood

+ Cutaneous: Vesicles and Eschar

+ Gastrointestinal: Stool and Blood

Bacillus anthracis Key Level A Lab Tests

+ Gram stain
+ Growth characteristics on agar
+ Sporulation, in air
+ Motility
+ (Penicillin-zone)*
+ (Capsule by India Ink)*
  - *optional

Bacillus anthracis Gram Stain Morphology

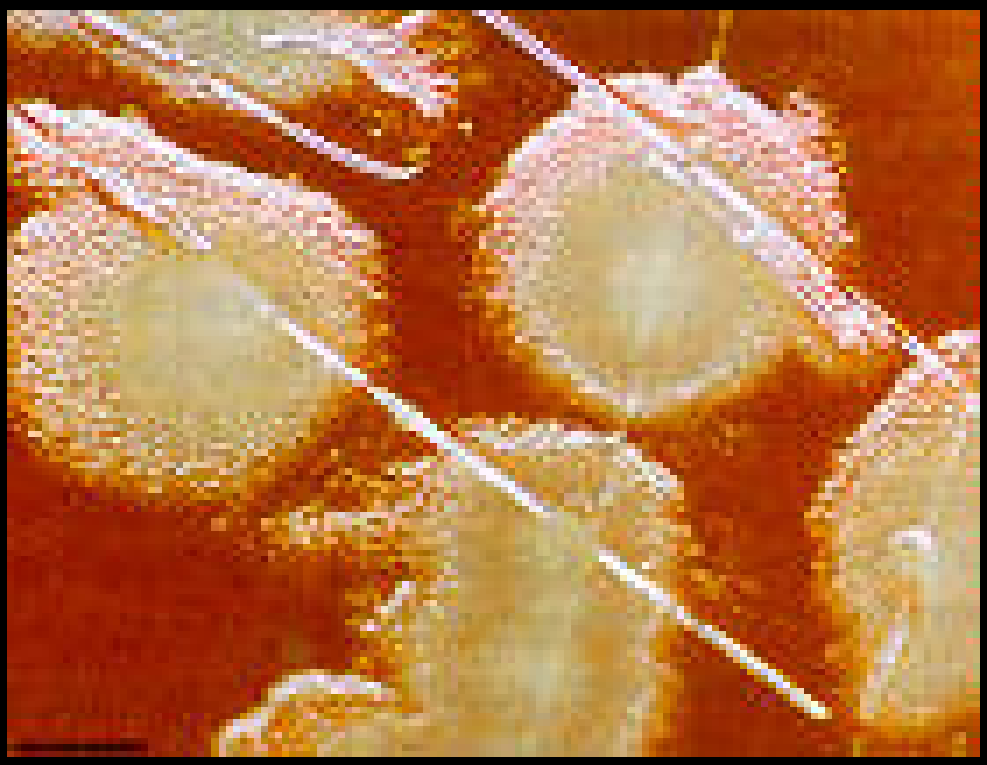
- Broad gram-positive rod: 1-1.5 X 3-5 µ
- Oval, central - subterminal spores: 1 X 1.5 µ with no significant swelling of cell
- Spores are NOT usually present in clinical specimens unless exposed to atmospheric O_2

B. anthracis, gram stain demonstrating spores

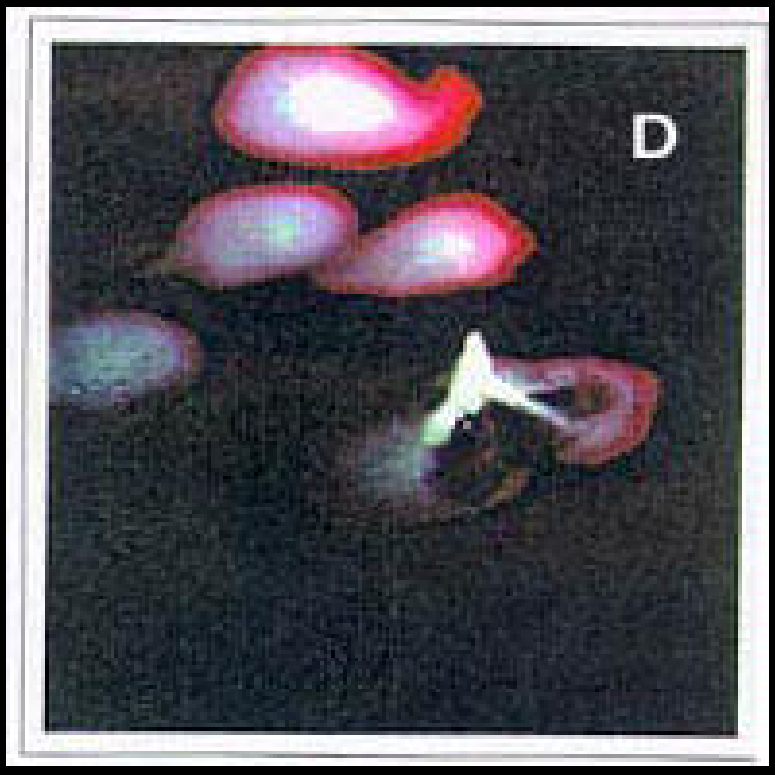
B. anthracis Colonial Morphology

- Colonial morphology of 18-24hr @ 35 C:
  - Well isolated colonies are 2-5 mm in diameter
  - Flat or slightly convex, irregularly round
  - Edges: slightly undulate, often curly tailing edges
  - Ground glass appearance
  - “Sticky” consistency….stands up like beaten egg whites

B. anthracis, colony on SBA



"STICKY" consistency of *B. anthracis'* colony on SBA



B. anthracis **on blood (left) and bicarbonate agar (right) plate (demonstrating capsule formation)**

Bacillus anthracis Presumptive Identification

- Gram-positive, broad rod, catalase-positive, spore-positive, aerobe: *Bacillus sp.*

- Spores are oval and nonswelling with ground glass colony appearance: Bacillus morphology group 1, includes *B. anthracis, B. cereus, B cereus var mycoides,* and *B. thuringiensis*

Bacillus anthracis Presumptive Identification, con't

- Nonmotile: *B anthracis* and *B cereus* var mycoides (and *B. megaterium*)
- Nonhemolytic, penicillin-zone (15-20 mm) and forms capsule: Presumptive *B. anthracis*

CDC Laboratory Pearl

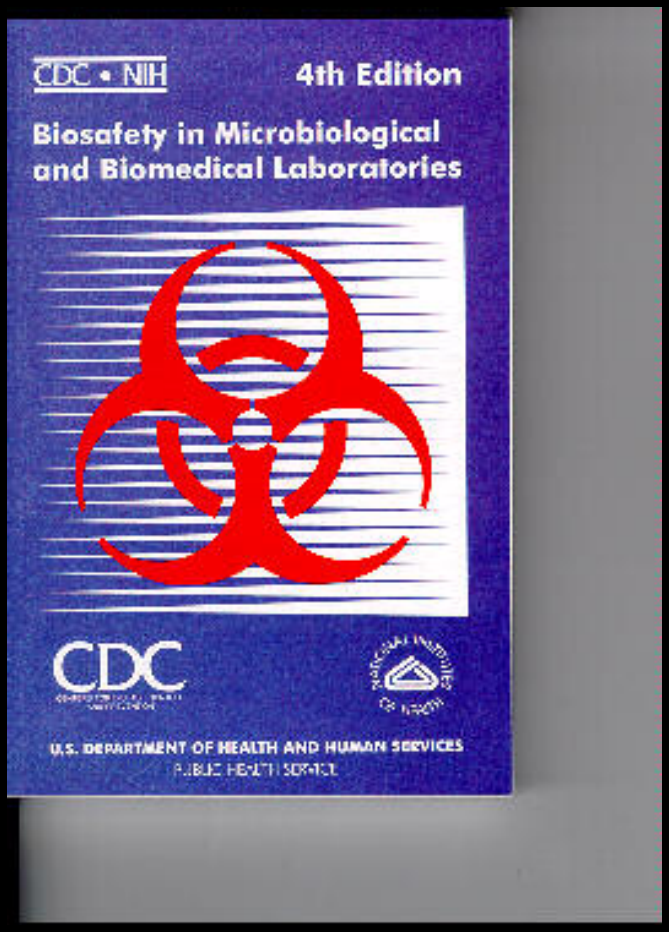
+ **The most common *Bacillus sp.* submitted to CDC to r/o *B. anthracis* are non-motile *B. megaterium***

+ **A non-motile, *Bacillus sp,* is recovered from a blood culture. What is one of the fastest procedures to r/o anthrax ?**

Bacillus anthracis TECHNICAL HINTS

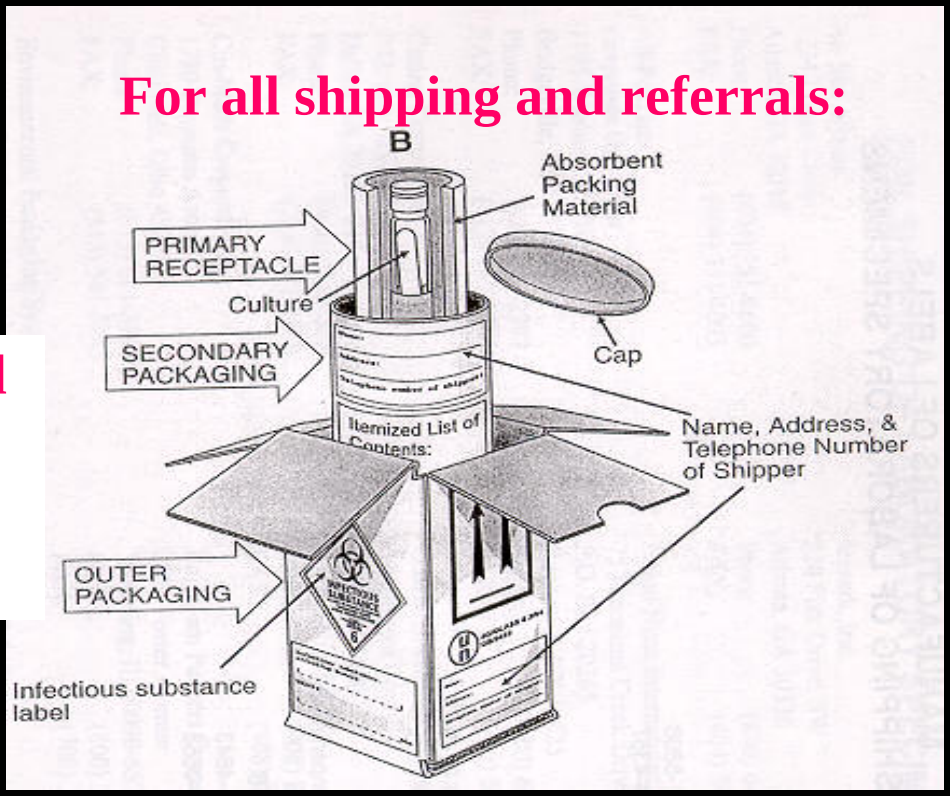
+ **Direct smears from clinical specimens:** encapsulated broad rods in short chains, 2 - 4 cells. India Ink will demonstrate capsule (gram stain will not)

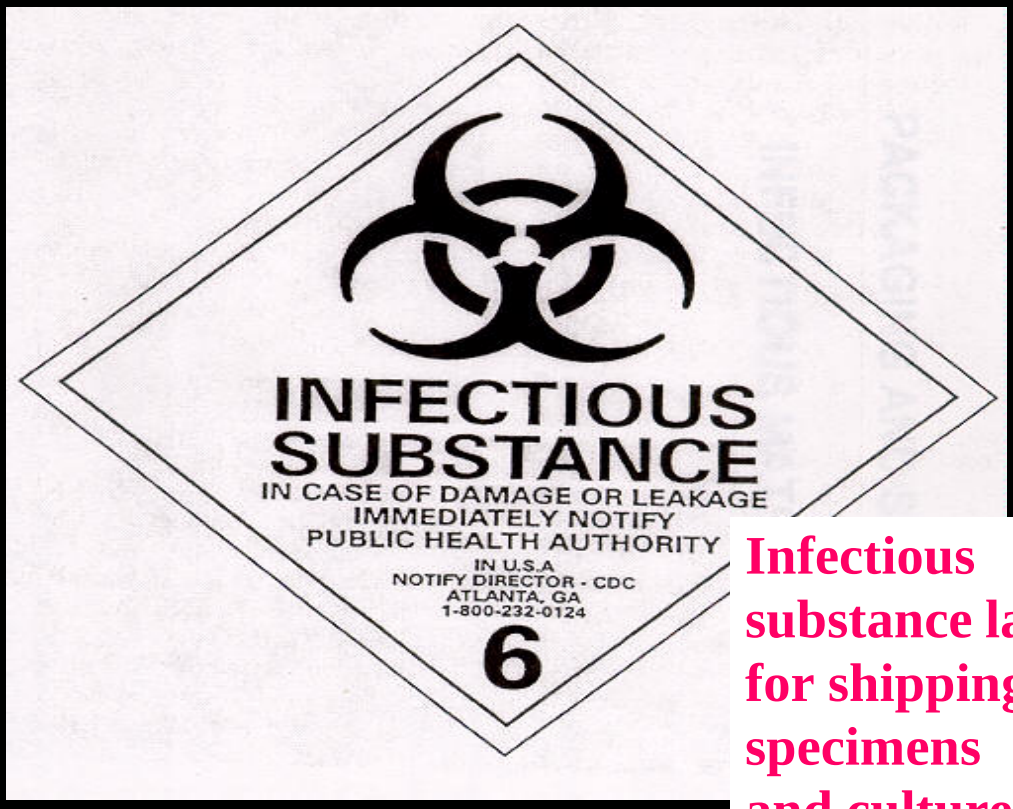
+ **Smears from SBA**: non-encapsulated broad rods in long chains



Packaging and Labeling of Infectious Substances

For all shipping and referrals:





Infectious substance label for shipping all specimens and cultures

Level A Laboratory Packaging/Shipping

+ Any clinical specimen (swabs, scrapings, body fluids, tissue) and/or “suspected” organisms that are referred to a higher level laboratory (B,C, or D) to rule out any of the agents discussed are to be classified as “INFECTIOUS SUBSTANCES”, and packaged and shipped accordingly.

Level A Laboratory Packaging/Shipping

+ All packaging must be UN certified for infectious substances, designated as: UN 4G/CLASS 6.2

+ NOTE: The above packaging is a “combination packaging” and must be used as a unit.

Level A Laboratory Packaging/Shipping

+ **Primary Receptacle: WATERTIGHT**
  - **Includes: glass, plastic, metal or screw-cap tubes.**
  - **Label: Specimen ID label**
  - **To ensure a leak-proof seal, screw caps must be fastened with tape, shrink seals or other comparable packaging**

Level A Laboratory Packaging/Shipping

- Absorbent material must surround each primary receptacle and be sufficient to absorb the contents of primary container

Level A Laboratory Packaging/Shipping

- Secondary Packaging: WATERTIGHT
  - Primary receptacle must be placed within the secondary packaging surrounded by absorbent material
  - Itemized list of contents, e.g., E. coli on 5ml slant, affixed to secondary packaging
  - Secondary package must be also be labeled with the following information: name, address, and telephone number of shipper

Level A Laboratory Packaging/Shipping

+ Outer Packaging: ADEQUATE STRENGTH
  – Secondary packaging (along with proper forms required from higher level laboratories) must be placed within the outer packaging unit

Level A Laboratory Packaging/Shipping

- Labels required on outer packaging:
  - Address label: higher level lab's address and phone number
  - Shipper's name, address and telephone number
  - Infectious substances label
  - Completed UN 2814 label

Level A Laboratory Packaging/Shipping

+ Overpack: ADEQUATE STRENGTH
  - A must with dry ice or cold packs
    - Dry ice must not be packed inside the combination packaging
  - Outer packaging (along with proper forms required from higher level laboratories) must be placed within the overpack
  - Labels required for overpack (see next slide)

Level A Laboratory Packaging/Shipping

- Overpack: ADEQUATE STRENGTH
  - Labels/forms required on overpack include:
    - Higher level lab's address and phone #
    - Shipper's name, address and telephone number
    - Infectious substances label
    - Completed UN 2814 label
    - "Inner packages comply with prescribed specifications"
    - Shipper's Declaration for Dangerous Goods form
    - Label for ice/dry ice (if used)
    - Contact shipping company for additional

Level A Laboratory Packaging/Shipping

SHIPPING CARRIER CRITERIA:

+ 1. Overnight delivery
+ 2. Package tracking capability
  - Such as: Airway Bill #

Websites of Interest

+ Biosafety in Microbiology Lab
  - www.cdc.gov/od/ohs/
+ Guideline for Isolation Precautions
  - www.cdc.gov/ncidod/hip/
+ Public Health Image Library
  - phil.cdc.gov/
+ WHO: zoonotic diseases
  - www.who.int/emc/